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COLOUR GENERALIZATION AND CHOICE BEHAVIOUR OF THE HONEYBEE *APIS MELLIFERA LIGUSTICA*

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Abstract—The colour choice of free-flying honeybees *Apis mellifera ligustica* was tested on a food-source simulator with 12 non-rewarded and coloured feeders (blue, violet or yellow) after training to rewarded feeders marked with aluminium discs. Bees trained to the aluminium signal showed a preference for violet followed by blue and finally by yellow. These results are interpreted using a model which allows a determination of the bee's perceptual distances between the stimuli used. The rank order obtained in colour-choice experiments can be related to generalization processes in which bees trained to the aluminium signal preferred the colour signal which is perceptually closest to the pretrained signal, even if innate colour preferences could not be discarded. Since bees generalize effectively from one visual cue to the other, it is to be expected that any pretraining would have a strong impact on the colour choice even if no obvious colour marks or "neutral" marks (aluminium discs) are used.

Key Word Index: Colour vision; honeybee; generalization process; colour preferences

INTRODUCTION

"Bees and various other insects must be directed by instinct to search flowers for nectar and pollen, as they act in this manner without instruction as soon as they emerge from the pupa state." In this way, Charles Darwin (Chap. XI, p. 425, 1877) anticipated, a hundred years ago, the concept of "innate search images" which could allow a pollinator to select a potential food source prior to any experience and then could also facilitate the choice behaviour after learning. The existence of preparedness for certain sensorial cues, in particular for visual cues, was clearly stated by Menzel (see 1985 for review). However, as predisposition is very difficult to demonstrate. Menzel used carefully controlled experiments based on learning processes as evidence for preparedness for some colours as food signals (Menzel, 1967, 1968). Nevertheless, information about this point has not yet been adequately analysed using the so-called spontaneous choice behaviour of free-flying honeybees. An important difficulty in such tests is the strong influence that would exerce the pretraining procedures which could obscure preexisting information. It has been claimed that foraging bees use the information learned on new places rather than transfer previous experiences of different places. If true, this fact would also underline the importance of pretraining procedures in relation to choice behaviour of experimental foraging honeybees.

We report here results concerning a rank order of colours as food signals, obtained from colour-choice-tests performed after controlled pretraining situations on an artificial device providing sugar solution on coloured feeders. Results are analysed as a consequence of differential generalization processes resulting from the pretraining situation, even if innate colour preferences could not be discarded.

MATERIALS AND METHODS

Apis mellifera ligustica from the apiary of the SADA (Argentine Beekeepers Society) were used. The hive was at a distance of 40 m from the laboratory.

We used a food-source simulator (Núñez, 1971; Giurfa and Núñez, 1989) placed in the laboratory on a table close to an open window. Bees were first trained to find sugar solution on the 12 numbered feeders of the simulator without colour marks on a grey background. The total flow rate supplied was 2.04 μl/min (each feeder received 1/12 of the total flow). With this flow supply, bees performed 117.5 ± 3.65 (mean ± SE; $n = 80$ visits) feeder inspections per visit. Thereafter, one bee was selected for measurements, marked with a colour spot on the thorax and trained during two visits to find the sugar solution on feeders marked with aluminium discs which reflected a continuous spectrum (Fig. 1). All the other bees were caged for later use. After training, bees were tested with two (Experiment 1) or three colours (Experiment 2) with an aleatory distribution in the food-source simulator. For this, two or three groups of feeders were previously defined (Groups I, II and III). On each feeder, the colour of the aluminium disc was placed between acrylic discs and

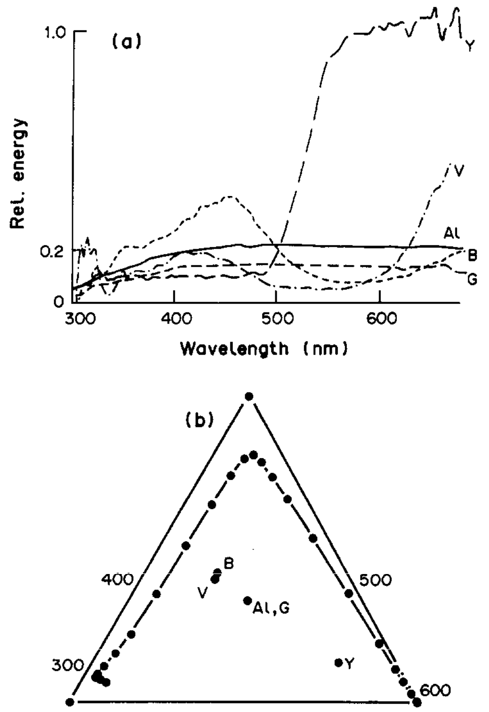

Fig. 1. (a) Spectral reflection functions of the four colour signals used. Al: aluminium; V: violet; B: blue; Y: yellow (G: grey background). The curves give the relative spectral reflection to a standard white ($BaSO_4$ pulver tablet). (b) Chromaticity diagram of the honey bee with the colour loci of the four colour signals used (after Menzel and Backhaus, 1989). Al: aluminium; B: blue; V: violet; Y: yellow (G: grey background). The three spectral receptor types contributing the three primary components for the calculation of the colour space are assumed to be adapted to the background of the experimental arrangement (the food source simulator) and the illumination is that of the standard daylight function D65. The spectral line is marked at 300, 400, 500 and 600 nm.

could not be marked by bees. In the test situation, feeders were not rewarded. Feeder choice (inspection of a feeder for sugar solution) was measured during the two training visits and during the five first minutes of the third visit, i.e. the test visit where feeders were not rewarded. Colours used were: violet, yellow and blue (Fig. 1).

To rule out the possibility that a change of chromatic properties of the aluminium discs was induced by the acrylic discs, the former were put over the later during training visits. Colour discs were maintained under acrylic discs in the test visits. Results showed no effect of acrylic discs on choice behaviour.

Colour parameters and reflection spectra were kindly determined by the laboratory of Professor Dr Randolf Menzel (Berlin, F.R.G.) and aluminium reflection spectrum by the Argentine Group of Colour (G.A.C.) from the National Institute of Industrial Technology, Buenos Aires.

Deviation from random distribution of inspections on two colours was tested with the "G-test" (Sokal and Rohlf, 1981). "χ^2-test" was used when three colours were present in the food source simulator, testing the null hypothesis of a random choice of the three colours employed. Colours were changed from one group (I, II or III) to another from bee to bee, each bee acting then as control from the others. Results were obtained by recording 120 visits of 40 bees.

Experiments were performed from February till May 1989, covering two different periods of nectar flow at natural food sources: summer and autumn.

RESULTS

In Experiment 1, an asymmetry in choices towards violet was observed when confronted with yellow (Table 1). The mean values for 13 bees tested show that bees making a symmetric choice of the two groups defined in training visits, were strongly biased to violet in the test visit ($G = 21.26$; $P < 0.001$). On the other hand, taking the test-visit values for each bee, a G-value of heterogeneity was obtained. In this case (violet vs yellow), $G_{\text{het}} = 16.28$ which is NS with 12 df; i.e. all the bees preferred in the same significant way violet when confronted with yellow.

Confronted violet vs blue, an apparent symmetry between the two colours was observed taking the mean values for 12 bees tested (Table 2: $G = 2.69$; NS). However, a G-value for the pooled data of the 12 replicates (violet: 398 inspections; blue: 251 inspections; $G = 33.59$; df = 1; $P < 0.001$) show that there is a pronounced deviation towards violet. Of the 12 bees tested, 5 evinced a significant G-value and 7 a non-significant G-value. Nevertheless, $G_{\text{het}} = 18.66$, NS with 11 df. That means that even if 7 bees showed non-significant G-values, in all the bees tested it existed a tendency towards violet when confronted with blue. No preference for blue was observed.

Finally, when comparing blue vs yellow, an asymmetry towards blue was evinced (Table 3: $G = 6.26$; $P < 0.025$). However, even if the G-value for the pooled data is clearly significant (blue: 211 inspections; yellow: 86 inspections; $G = 54.28$; df = 1; $P < 0.001$), in this case $G_{\text{het}} = 79.94$, also significant with 7 df ($P < 0.001$). That is, not all the bees showed a clear deviation towards blue, confronted with yellow, even if the mean values or the data as a whole gave place to significant bias towards blue.

Experiment 2 confirmed the ranking established between the three colours with violet in first, blue in second and yellow in the third place. Bees that make a symmetric choice between the three groups of

Table 1

	Visit	Group I	Group II	Total	G
Training	1	53	56	109	0.08 (NS)
	2	61	61	122	0 (NS)

Test—violet: 38; yellow: 8; total: 46; $G = 21.26$ ($P < 0.001$).

Violet vs yellow (n = 13 bees). Mean values of feeder choice for 13 bees tested with violet vs yellow signals on feeders (Test), after two training visits with aluminium discs on feeders.

In the test situation, colours were assigned to Group I and II (six feeders each) ensuring an aleatory distribution in the food-source simulator.

Table 2

	Visit	Group I	Group II	Total	G
Training	1	49	48	97	0 (NS)
	2	60	58	118	0.02 (NS)

Test—violet: 33; blue: 21; total: 54; $G = 2.69$ (NS).

Violet vs blue (n = 12 bees). Mean values of feeder choice for 12 bees tested with violet vs blue signals on feeders (Test), after two training visits with aluminium discs on feeders.

In the test situation, colours were assigned to Group I and II (six feeders each) ensuring an aleatory distribution in the food-source simulator.

Table 3

	Visit	Group I	Group II	Total	G
Training	1	43	49	92	0.39 (NS)
	2	61	61	122	0 (NS)

Test—blue: 26; yellow: 11; total: 37; $G = 6.26$ ($P < 0.025$).

Blue vs yellow (n = 8 bees). Mean values of feeder choice for 8 bees tested with blue vs yellow signals on feeders (Test), after two training visits with aluminium discs on feeders.

In the test situation, colours were assigned to Group I and II (six feeders each) ensuring an aleatory distribution in the food-source simulator.

feeders previously defined in training visits, showed, in the test situation, a significant bias towards violet, being blue in the next place more preferred than yellow (Table 4: $\chi^2 = 11.77$; $df = 2$; $P < 0.005$).

DISCUSSION

Our results suggest, after pretraining to a quite evenly reflecting signal that a rank order of choice of colours as food signals can be established in free-flying honeybees. Experiment 1 allows for a comparison between pairs of colours and Experiment 2 confirms the rank order established in Experiment 1. Bees trained to a stimulus with a spectral reflection of about an even amount over the spectrum and a higher reflection in the ultra violet than the background, showed thereafter a preference for violet, followed by blue and finally by yellow. It seems difficult to decide whether this biased behaviour is the consequence of innate spontaneous colour preferences (Menzel, 1967, see also Menzel, 1985 for review) or results from differential generalization processes after training to the aluminium signal. In the later case, an estimation of the perceptual distances between the stimuli used is necessary. An appropriate determination of these perceptual distances is possible using the model stated by Backhaus *et al.* (1987a) and Menzel and Backhaus (1989) who found that the two perceptual dimensions defining the colour space of the bee correspond to a two colour opponent processes: ultra violet/blue-greenness and blue/ultra violet-greenness, respectively. The total perceptual colour difference is related to the differences on these two axes by the city-block metric, i.e. in this model, the perceptual distance is calculated as the sum of the absolute distances on the scales A and B given in Fig. 2 (scale A: ultra violet/blue-greenness; scale B: blue/ultra violet-greenness). From Fig. 2 (calculated for a background adaptation on the grey used), one can obtain the perceptual distances D between the loci of our stimuli as:

$$D = |(A_i\text{-}A_{\mathrm{Alu}})| + |(B_i\text{-}B_{\mathrm{Alu}})|,$$

where A_i and B_i are the coordinates of colour i on the A and B scales, respectively.

The distances D are: between aluminium and violet, 2.39; between aluminium and blue, 2.716 and between aluminium and yellow, 5.90. Then, the rank order obtained in colour choice experiments can be related to a generalization process in which bees trained to the sanded aluminium signal prefer in a significant way the colour signal which is closest to the pretrained signal. Considering the distances established between the aluminium signal and yellow and blue signals, one can also understand the colour choice results as a consequence of such a generalization process. Even if blue and violet are very near one of each other, the differences observed could be related to the slight differences in distances to the aluminium locus. Yellow, the more distant from this locus, would be then the less frequently preferred.

In the choice situation that gives place to the model of Backhaus *et al.* (1987a) (see also Menzel and Backhaus, 1989), brightness is ignored by bees in colour-training experiments where the bees are not particularly trained to brightness differences of signals with no or very little differences in hue and saturation (see also Backhaus *et al.*, 1987b). In fact, this coincides well with the results of Daumer (1956) and von Helversen (1972). In this context, brightness is defined as the sum of the absorptions on the three spectral receptor types after its adaptation to the background screen in the experiment. However, bees can perceive brightness differences of colour signals as was shown in early experiments by von Frisch (1914) and in phototaxis experiments (Menzel and Greggers, 1985). Then, our results could be biased by spontaneous preferences related to this dimension; i.e. bees alight on violet not for violet itself and its

Table 4

	Visit	Group I	Group II	Group III	Total	X^2
Training	1	39/39	39/39	39/39	117	0 (NS)
	2	37/36.33	36/36.33	36/36.33	109	0.02 (NS)

Test—violet: 21/13.33; blue: 13/13.33; yellow: 6/13.33; Total: 40; $X^2 = 11.77$ ($P < 0.005$).

Violet vs blue vs yellow (n = 7 bees). Mean values of feeder choice for 7 bees tested with violet vs blue vs yellow signals on feeders (Test), after two training visits with aluminium discs on feeders. Under bars the expected frequencies for each group.

In the test situation, colours were assigned to Group I and II and III (four feeders each) ensuring an aleatory distribution in the food-source simulator.

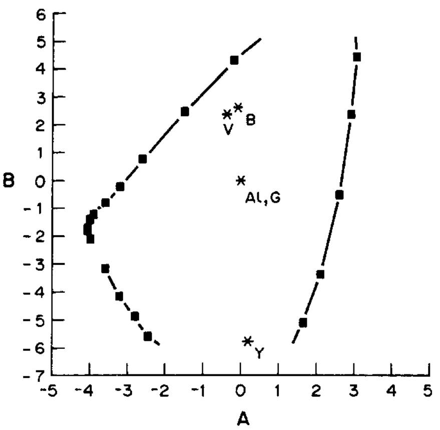

Fig. 2. Colour opponent diagram after Menzel and Backhaus (1989), including the colour loci of the four colour stimuli used. Al: aluminium; B: blue; V: violet; Y: yellow (G: grey background). As in Fig. 1(b), the receptors are assumed to be adapted to the background under natural daylight conditions (standard function D65). The two axes A and B correspond to the two colour opponent processes ultra violet/blue-greenness and blue/ultra violet-greenness, respectively (see text).

proximity to the aluminium locus, but for a special brightness characteristic. However, measurements of the colours employed allow us to establish a brightness-rank order: (1) blue: 1.3411; (2) yellow: 1.2266; (3) violet: 1.0114, a rank order which does not correlate with the rank order of colour choice observed.

We cannot exclude the influence of innate colour preferences on our results, considering that our rank order agrees with the rank of preparedness proposed by Menzel (1967) in learning tests: violet (400–420 nm) is learned fastest and is chosen most accurately, followed by blue, yellow, green, ultra violet and finally by bluish-green which is learned most slowly and chosen with least precision; in addition, blue has a larger retention interval than yellow (Menzel, 1968) which agrees with the rank order of acquisition experiments. However, differential generalization processes can explain for itself the results obtained by us.

Colour preferences could be adaptive traits related to colour more frequent on the biotope explored by bees. However, for this question, we have to use autochthonous bee populations selected for different biotopes, a condition not satisfied in our situation: honeybees used by beekeepers are not indigenous of South America as well as the floral composition of fields exploited by bees in the surrounding of Buenos Aires.

External ecological conditions could have influenced the choice behaviour of the bees tested, i.e. the overall balance of nectar flowers—and their colours—in the surrounding, could be reflected in the choice behaviour observed. However, all the 40 bees tested gave concordant results on experiments covering two different periods of sugar flow in food sources (summer and autumn).

The main conclusion of our results is that pretraining plays a decisive role in the so-called spontaneous choice behaviour. Bees choice colour signals in relation to previous experience learned on the artificial food sources rather than transfer learned information from any learning at different places. Even if colours tested are not available during pretraining, differential generalization processes are possible and can strongly influence any spontaneous choice behaviour. As it was said by Menzel (personal communication), "any learning trial appears to whipe out the old memory and induce a search behaviour which is guided by the learning trial at the new place."

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